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(54) Title: COMPOSITIONS AND METHODS FOR ENHANCING THE GROWTH OF HAIR AND RESTORING HAIR COLOR

(57) Abstract

The present invention relates to a method for stimulating dormant hair follicles in intact skin, especially intact skin of the scalp of humans and enhancing the growth and restoring the natural hair color of hair which has been diminished in its natural color. The formulations according to the present invention are useful for increasing hair growth by stimulating dormant, weak of dying hair follicles to produce hair. Auxiliary to such hair growth is the unexpected result that the hair's natural color is restored to hair which is diminished in its natural color, for example in graying and/or gray hair, as the hair grows. In addition, the formulations act to produce angiogenesis in epidermal tissue surrounding hair follicles as well as enhance the growth of ungual tissue or revitalize skin in animals. These formulations generally comprise an effective amount of a mixture of non-steroidal anabolic hormones selected from insulin, growth hormone, triiodothyronine or thyroxine, in combination with a minimum essential medium, preferably, an enriched minimum essential medium such as MCDB 153.

COMPOSITIONS AND METHODS FOR ENHANCING THE GROWTH OF HAIR AND RESTORING HAIR COLOR

FIELD OF THE INVENTION

The present invention relates to methods for stimulating hair follicles and promoting the growth of hair in intact skin, especially the scalp. More particularly, the invention relates to a method for inducing angiogenesis and causing increased vascularization and increased circulation (vasodilation) of intact skin tissue and stimulating dormant, dying or weak hair follicles to produce hair or promote the growth of hair in such skin and reduce hair loss. The present invention also relates to a method for stimulating nail growth and producing stronger nail (ungual) tissue. In addition, the present invention also relates to a method for promoting melanogenesis and restoring the natural color to hair in which the color is diminished or diminishing, such as in gray or graying hair. The compositions of the present invention which are used to enhance hair and nail growth and promote melanogenesis and restore natural hair color are compositions which are based on a minimum essential medium in combination with preferably at least two non-steroidal anabolic hormones one of which hormones is insulin and the other which is selected from triiodothyronine, thyroxine and growth hormone. Three anabolic hormones, including insulin, triiodothyronine or thyroxine and growth hormone in combination with minimum essential media may be preferably used. Preferably, an enriched growth media such as MCDB 153 or related enriched growth media is used in combination with insulin, triiodothyronine or thyroxine and growth hormone. Compositions for promoting hair and nail growth may also contain an effective amount of a penetration enhancement agent for promoting the penetration of the active constituents of the compositions through the surface of the skin where angiogenesis, melanogenesis and vascularization may be promoted.

which no cell proliferation occurs. The factors that regulate cell division are poorly understood, although growth factors, steroid hormones, dermoepithelial interactions and the immune system have been implicated. Philpott, et al., J. Cell Sci., 97(Pt3), 463 (1990), recently demonstrated that growth factors such as epidermal growth factor (EGF) mimic the in vivo depilatory action of EGF resulting in the formation of a club hair-like structure; and that transforming growth factor (TGF)-β_1 may serve as a negative growth regulatory factor for the hair follicle.

One area of investigation into hair growth involves the use of androgens (steroidal hormones) where it has been shown that androgens modulate the type of hair produced by hair follicles in humans.

A number of recent publications evidence that human scalp cells and hair may be grown in vitro. Several publications report the use of hormonally supplemented serum-free cell culture medium of the MCDB series as an optimal medium for growing human hair cells in culture. Kitano, et al., J. Dermatol., 19(11), 793, (1992), used MCDB 153 supplemented with amino acids, hydrocortisone, insulin, EGF and bovine pituitary extract to grow isolated human scalp hair follicles in vitro. Tanigaki, et al., Arch. Dermatol. Res., 282(6), 402, (1990), used MCDB supplemented with 7 growth factors to induce hair cell differentiation of C3H mice hair cells in culture. M.P. Philpott, et al., Ann.NY Acad. Sci., 642, 148 (1991), reported that serum inhibited hair follicle cell growth in vitro while serum-free medium supported its growth. Tobin, et al., Arch. Dermatol. Res., 285(3), 158 (1993), maintained human hair follicles in culture in serum-free medium.

The main source of energy for hair follicle cells in the culture medium was reported by Williams, et al., J. Invest. Dermatol., 100(6), 834 (1993). That group observed that the glucose-glutamin cycle supports human hair follicle growth rate. Philpott, et al., J. Cell Sci., 97(Pt3), 463 (1990), demonstrated that growth factors such as TGF-beta in vitro and TGF-alpha and EGF in vivo had negative effects on

hair growth was established. Still other studies have shown that keratin growth factor may stimulate hair follicles as well as sebaceous glands.

The importance of the vascularization of the dermis to the process of hair growth is well known. Thus, any factor which negatively affects the skin microvasculature will most likely also undermine hair growth.

The presently available product Minoxidil^{tm} has been shown to be efficacious in enhancing hair growth. Its mechanism of action is based upon its vasodilatory effect which causes shifting of the fluid volume from the vascular compartment into the extracellular compartment, especially in the dermal area. This, in turn, may cause an increase in blood circulation in the dermis and increased supply of nutrients to the dermis affecting the hair follicles.

In experiments on wound healing, MCDB 153 supplemented with non-steroidal anabolic hormones was used as treatment on surgical wounds. The skin on the dorsum of all the animals was depilated prior to the extirpation of the skin patches. During the change of bandages it was noted that the depilated hair on the skin adjacent to the wound edges was growing at a faster rate than the hair on areas further away from the treated wound. The application of MCDB 153 including anabolic hormones induced vascularized granulation tissue formation as well as epithelialization. The barrier for penetration through the skin, the keratinized layer, was overcome by the opening in the wound area. This condition allows for unhindered contact between the medium and the deeper layers of the skin including the hair follicle cells and the capillary endothelial cells.

Unpublished data by the present inventor on angiogenic activity of supplemented MCDB 153 (with insulin, growth hormone and triiodothyronine or thyroxine) performed on the CAM (chorioallantoic membrane) indicated that the application of the gelled medium did not elicit vascular response. The gel did not appear to penetrate the external epithelial layers

promoting melanogenesis in hair follicles and for restoring the hair color in hair which exhibits diminished hair color such as in gray, graying and damaged hair.

The present formulations comprise a hair or nail growth enhancing effective amount of a non-steroidal anabolic hormone selected from insulin, growth hormone, triiodothyronine and thyroxine (T_3 or T_4), and mixtures thereof, most preferably a mixture of all three hormones, in combination with a hair or nail growth enhancing effective amount of minimum essential medium, preferably a supplemented medium such as MCDB 153. The formulations may be adjusted to enhance penetration of the individual components through intact skin in order to promote angiogenesis of the underlying dermal layers and consequently, to stimulate hair follicles and ungual tissue and promote hair or ungual tissue growth. All of the components used in the compositions according to the present invention are included in amounts effective for an intended use generally- enhancing or stimulating hair and/or nail growth or restoring hair color in hair exhibiting diminished color.

In preferred embodiments according to the present invention, the non-steroidal anabolic hormone is a mixture of insulin and at least one or more anabolic hormones such as triiodothyronine, thyroxine and growth hormone. It has been unexpectedly discovered that a mixture of the anabolic hormones insulin and triiodothyronine, thyroxine or growth hormone produces a synergistic enhancement in hair growth when combined with a minimum essential medium. In more preferred embodiments according to the present invention, the anabolic hormone comprises a mixture of an amount of insulin, growth hormone and triiodothyronine or thyroxine in amounts effective to substantially enhance the growth of hair or ungual tissue, also synergistically. Embodiments in which the anabolic hormone is a mixture of effective amounts of triiodothyronine or thyroxine and growth hormone or insulin and growth hormone are also contemplated by the present invention. Generally in concert with enhanced hair growth, hair color restoration occurs in hair which is diminished in hair color. Composi-

The preferred amount of anabolic hormone other than insulin used will generally depend on the extent and rate of hair or nail growth desired or damage to hair, nail or skin tissue which is to be corrected, but in most of the cases the amount of hormone will fall within a preferred range of about 0.5 ng/ml and about 100 ng/ml by weight or more of the composition. In the case of compositions which are delivered as a gel, growth hormone, preferably human growth hormone, is included in an amount ranging from about 0.5 ng/ml to about 50 ng/ml by weight or more, more preferably about 0.5 ng/ml to about 10 ng/ml. Triiodothyronine (T_3) or thyroxine (T_4) is preferably included in amounts ranging from about 0.5 ng/ml to about 100 ng/ml or more. Triiodothyronine (T_3) may be preferred over thyroxine (T_4) because it has greater potency and the same general activity as thyroxine. Thyroxine (T_4), however, is more storage stable than is triiodothyronine (T_3) and thyroxine's stability should be taken into account and is preferred when formulating compositions which are to be stored for at least several weeks or more. Triiodothyronine (T_3) and thyroxine (T_4) may be readily substituted for each other, however, with the general rule that at least about three to five times the amount of thyroxine (T_4) is substituted for triiodothyronine (T_3).

BRIEF DESCRIPTION OF THE FIGURES

Figures 1-2 represent the results of the experiments performed and described in Example 3. These graphs show the relative degree of increase in blood flow measured by Laser-Doppler Flow changes on burn wounds treated with a composition of the present invention and conventional therapy (Silverol).

DETAILED DESCRIPTION OF THE INVENTION

In describing the present invention in the specification, a number of terms will be used.

The term "angiogenesis" is used throughout the specification to describe processes which result in the development of new blood vessels and the vasculature (neovasculature).

in the hair gel compositions according to the present invention, with hydroxyethylcelllulose being more preferred. One of ordinary skill in the art will recognize to vary the type and amount of delivery polymer in compositions according to the present invention to provide enhanced delivery of the hair growth composition appropriate for topical delivery and penetration of the individual components through intact skin. The term delivery polymer is also used to describe polymers which instill slow-release or sustained release characteristics to the hair and nail growth formulations of the invention.

The term "minimum essential medium" is used throughout the specification to describe a medium or mixture which contains no serum, and in combination with anabolic hormone and optionally, a penetration enhancement agent, comprises the compositions according to the present invention. The term minimum essential medium is readily understood by those in the art to comprise a nutrient media which supports cellular growth. The minimum essential medium according to the present invention preferably comprises the following elements: (a) essential amino acids; (b) non-essential amino acids; (c) vitamins selected from the group consisting of biotin, folate, lipoate, niacinamide, pantothenate, pyridoxine, riboflavin, thiamin and vitamin B_{12} and mixtures thereof, preferably a vitamin mixture comprising folate, niacinamide, pantothenate, pyridoxine, riboflavin and thiamin; (d) glucose; and (e) a mixture of inorganic ions selected from the group consisting of calcium, sodium, potassium, magnesium, chloride and mixtures thereof, preferably a mixture comprising calcium, sodium, potassium, magnesium and chloride. Optionally, vitamin C may be included as one of the preferred vitamins in the present formulations. It is noted that a minimum essential medium for use in the present invention may exclude non-essential amino acids (b), but preferably, non-essential amino acids are included in combination with essential amino acids. Especially preferred amino acids include glutamine, serine and cysteine.

All of the above-described elements (a), (b), (c), (d) and (e) are included with the anabolic hormone mixture in con-

and (j), the concentration preferably ranges from about 1 umol. to about 50 mmol. One of ordinary skill in the art will be able to readily modify the type and amount of the components of the minimum essential medium consistent with the teachings of the present invention.

In addition to serum free minimum essential medium, the present invention may also make use of medium containing serum, although the use of a serum containing cellular nutrient medium is generally less preferred than is serum free medium. Examples of such nutrient medium include, among numerous others, DMEM, HAM F12 and HAM F10, all containing serum. The term "minimum essential medium" is used throughout the specification to describe all types of nutrient medium contemplated for use in the present invention which contain at least the basic elements as described hereinabove, as well as supplemental components.

MCDB 153 is a preferred supplemented minimum essential medium for use in the present invention. MCDB 153 is believed to contain the components which are associated with an enhanced rate of hair growth as well as other components which enhance the rate of angiogenesis and vascularization of tissue surrounding the hair follicle and consequently, the growth of hair from the follicle and, in many instances, the restoration of natural hair color in growing hair.

The minimum essential medium according to the present invention may include one or more commercially available media in solution or lyophilate (solid) form. The cellular nutrient medium used may be in the form of a lyophilate which may be reconstituted with water, preferably sterilized, distilled water and then supplemented with an anabolic hormone such as insulin, triiodothyronine, thyroxine, growth hormone or mixtures thereof, and optionally, certain penetration enhancing agents or other additives. Lyophilized forms of insulin, growth hormone and triiodothyronine or thyroxine may also be used in the present compositions. Alternatively, the medium may be used directly in formulations according to the present invention in the form of a lyophilate, or related solid-type

hormone and triiodothyronine or thyroxine is preferably used because a combination of non-steroidal anabolic hormones and minimum essential medium creates an enhancement of tissue growth (hair, ungual tissue, skin revitalization) which is greater than the sum of the individual parts. Three anabolic hormones (insulin, triiodothyronine or thyroxine and growth hormone) in effective amounts in combination with minimum essential medium acts synergistically to promote angiogenesis, vascularization and consequently, hair growth and in many instances, melanogenesis and hair color restoration. Thus, it is believed that the non-steroidal anabolic hormone actually enables the cells to utilize or process the nutrients in the media, which action results in angiogenesis, and an enhancement of vascularization and rate of hair and nail growth and skin revitalization. Restoration of hair color is an auxiliary beneficial result which occurs along with enhanced hair growth in many instances.

The term "penetration enhancement agent" is used throughout the specification to describe compounds which are used to treat the skin before or during treatment with the hair or nail growth promoting compositions according to the present invention or which are added to the other components in the present formulations in amounts effective to enhance the penetration through the skin of the non-steroidal anabolic hormones and the individual components of the minimum essential medium. It is noted that compositions according to the present invention may be used with or without the inclusion of a penetration enhancement agent. It is an unexpected result that compositions which exclude a penetration enhancement agent will be absorbed into intact skin and evidence a surprising degree of activity (i.e., hair or nail growth enhancement or skin revitalization). While not being limited by way of theory, it is believed that the components used in the present composition are absorbed through the skin by way of hair roots and pores in the skin in a manner sufficient to evidence significant activity.

Exemplary penetration enhancement agents which may be used to treat the skin before applying the present formula-

In addition to the above agents, a negatively charged synthetic membrane (which generates a static charge) may also be used for enhancing the individual components in compositions according to the present invention.

The amount of each penetration enhancement agent which is used in the formulations according to the present invention will depend upon the requirement for hair or nail growth or skin revitalization, but each component is included in an amount effective for producing the intended results, for example, substantially enhancing the rate of penetration of the formulations through the skin. This amount will vary according to the type of agent used. One of ordinary skill in the art will know to vary the amount and type of agent within the weight ranges defined above to enhance penetration through intact skin and promote the efficacy of compositions according to the present invention.

In general, in embodiments according to the present invention, the formulations include an anabolic hormone other than insulin at a concentration of at least about 0.05 ng/ml, preferably about 0.5 ng/ml to about 100 ng/ml or more. In the case of formulations containing insulin, the amount of insulin generally falls outside of this range. Preferably, the anabolic hormone comprises a mixture of insulin, triiodothyronine or thyroxine and growth hormone because of the known benefits these hormones have in promoting the growth and elaboration of cells and their general absence of toxicity. In addition, it is this combination of anabolic hormones which evidences unexpected synergistic activity in promoting angiogenesis, vascularization and hair or nail growth in the instant invention.

The preferred insulin is human insulin (more preferably human recombinant or genetically engineered insulin), which is a well-known protein which is readily available commercially from a number of sources (for example, Novo Nordisk, Copenhagen, Denmark, among others). It is constituted from a number of amino acids (approximately 51) with a total molecular weight of about 5,500. Human insulin for

triiodothyronine is more potent than is thyroxine but is less stable. Where thyroxine is substituted for triiodothyronine, to obtain the same effect as triiodothyronine, thyroxine is added in an amount between about three and five times that of triiodothyronine. Thyroxine, being more stable, is preferred for use in the present invention in those compositions which advantageously have storage stable characteristics. While not being limited by way of theory, it is believed that the triiodothyronine or thyroxine utilized in the present invention stimulates vascularization and facilitates the resupply of blood borne components in cells responsible for the growth of hair.

A particularly preferred composition according to the present invention comprises a mixture of an effective amount of human growth hormone in the presence of an effective amount of insulin and triiodothyronine (T_3) or thyroxine (T_4), preferably in a serum free cellular nutrient medium, most preferably MCDB 153. In this preferred embodiment of the instant invention, the anabolic hormones other than insulin, i.e., growth hormone or triiodothyronine, are generally included in the final composition in a concentration range of about 0.05 ng/ml to about 100 ng/ml, preferably about 0.5 ng/ml to about 20 ng/ml or more and most preferably about 1 ng/ml to about 20 ng/ml. In the case of the inclusion of thyroxine as a substitute for triiodothyronine, thyroxine is generally included in an amount ranging from about 0.5 ng/ml to about 100 ng/ml or more, generally at a concentration of at least about three-five times that of triiodothyronine.

In the most preferred embodiment which includes three anabolic hormones, insulin is also included in an effective amount, generally an amount which is substantially greater than the other anabolic hormones. The amount of insulin is preferably included in amounts ranging from about 5ng/ml to about 100 ug/ml more preferably about 50 ng/ml to about 20 ug/ml and even more preferably about 500 ng/ml to about 20 ug/ml. One of ordinary skill in the art will know to vary the amount of anabolic hormones within effective ranges based upon the type and potency of the preparation of the compound in

amino acids (b) are preferably added, but are not required. The preferred serum free nutrient medium is modified MCDB 153, a well-known medium. Mixtures of standard commercial nutrient media may also be used with favorable results in the instant invention.

While not being limited by way of theory, it is believed that one plausible explanation of the mechanism of the accelerated growth of hair and nails, hair color restoration and skin revitalization is that the presence of the anabolic hormones, and in particular, the combination of insulin, triiodothyronine or thyroxine and human growth hormone in the formulations according to the present invention, synergistically promotes the utilization of the nutrients from the nutrient medium and consequently, the promotion of angiogenesis and melanogenesis and vascularization of the tissue surrounding the hair follicle and nail growth tissue. The result is the stimulation of hair follicles and related nail tissue and consequently enhanced rate of growth of hair and nails and hair color restoration. In addition, not only is the rate of growth of hair enhanced, but also the number, quality and in most instances, melanogenesis of active hair-growing follicles growing hair also increases, primarily due to the stimulation of dormant, dying or weak follicles to produce hair. In effect, the quality of the growth of hair, nail and skin tissue is enhanced using the compositions of the present invention. Hair color restoration is an auxiliary effect.

With regard to the enhancement in angiogenesis and the promotion of vascularization, the mechanism which might be assumed is that new capillaries appear in the tissue surrounding the hair follicles and related nail tissue from the first day on and reach their maximum levels after one week or so. The new vessels in granulation tissue originate as budlike structures on nearby vessels, enhance vascularization, become canalized and ramify throughout the dermis in proximity to the hair follicles and nail growth tissue. The resulting increase in vascularization provides more nutrition and stimulatory factors for the hair follicle and nail and skin tissue, the

tained in the serum.

While a large number of serum free nutrient media may be used in the present invention, a preferred nutrient media for use in the present invention is modified MCDB 153.

Hereafter are enumerated the particular constituents and concentrations of the above groups for the preferred medium, MCDB 153:

Group (a):	Concentration (M)
Arginine	1.0 x 10-3
Cysteine or Cystine	2.4 x 10-4
Glutamine	6.0 x 10-3
Histidine	8.0 x 10-5
Isoleucine	1.5 x 10-5
Leucine	5.0 x 10-4
Lysine	1.0 x 10-4
Methionine	3.0 x 10-5
Phenylalanine	3.0 x 10-5
Threonine	1.0 x 10-4
Tryptophan	1.5 x 10-5
Tyrosine	1.5 x 10-5
Valine	3.0 x 10-4
Group (b):	
Alanine	1.0 x 10-4
Asparagine	1.0 x 10-4
Aspartate	3.0 x 10-4
Glutamate	1.0 x 10-4
Glycine	1.0 x 10-4
Proline	3.0 x 10-4
Serine	6.0 x 10-4
Group (c):	
Biotin	6.0 x 10-8
Folate	1.8 x 10-6
Lipoate	1.0 x 10-6
Niacinamide	3.0 x 10-7
Pantothenate	1.0 x 10-6

may be varied within the concentrations described hereinabove (in hair or nail growth enhancing effective amounts) to provide formulations workable within the description of the present invention.

Preferably, the non-steroidal anabolic hormone to be incorporated into the modified MCDB 153 composition, according to the present invention, is a mixture of at least two hormones selected from insulin, triiodothyronine/thyronine and growth hormone at hair growth enhancing effective concentrations. Most preferably, the anabolic hormone includes a mixture of human growth hormone, insulin (containing transferrin or transferrin-free) and triiodothyronine (T_3) or thyroxin (T_4), each hormone included in a hair growth enhancing effective amount. The three hormone combination exhibits an unexpected synergistic effect in promoting hair growth. Hormones other than insulin are included in an amount ranging from at least about 0.05ng/ml, preferably at least about 0.5ng/ml to about 100 ng/ml, and more preferably about 1 ng/ml to about 100 ng/ml. In the case of thyroxine, it is generally substituted for triiodothyronine at a concentration of at least about three to five times the concentration of triiodothyronine used. In the case of insulin, the effective amount of insulin generally ranges from about 5ng/ml to about 100ug/ml and more preferably about 50 ng/ml to about 20ug/ml, even more preferably about 500 ng/ml to about 20 ug/ml within this range.

Insulin is a desirable constituent anabolic hormone, found to impart a maturing stimulus of the growing culture. Insulin may be commercially obtained and is generally provided in mU quantities (about 41 ng of insulin). The International Unit of Insulin (SI= System International) is the activity contained in 0.04167 mg (41.67 ug) of the 4th International Standard Preparation (1958). The Standard Preparation is a quantity of purified Zinc Insulin crystals extracted 52% from Bovine and 48% from Porcine pancreas (See, Martindale Pharmacopoeia, 26th Ed.).

In addition to effective amounts of non-steroidal

polyethylene, acrylamide, polyacrylamide, amylose or collagen to promote the delivery of the components to the surface of the skin. The inclusion of a cellulose ether gellation agent and in particular, the use of methyl cellulose or hydroxyethyl cellulose, is clearly preferred. In general, the amount of delivery polymer which is added to the formulations to produce a gel is that amount which solidifies the composition to a point where the composition does not easily flow off of the intact skin to be treated and generally ranges from about 0.1% by weight to about 20% by weight, preferably about 0.5% to about 10% by weight, depending upon the type of delivery polymer used. The gel compositions according to the present invention preferably contain sufficient water or moisture to maintain the area to be treated in a relatively moist state- a condition shown to be beneficial for penetration enhancement of the individual components of the formulations through the skin.

In addition to solution, gel or hydrogel forms, compositions according to the present invention also may be formulated as creams, elixirs, powders and the like for delivery to the scalp or other area of intact skin to be treated for the hair growth enhancing effects of the present compositions. The various components of the compositions according to the present invention may have to be varied in order to maintain effective concentrations. When compositions according to the present invention are formulated, these compositions may also contain an amount of a pharmaceutically acceptable excipient and, in addition, other additives such as diluents, compounding agents, bulking agents, surfactants and the like. One of ordinary skill in the art will recognize to vary the concentrations of the individual components as a function of the type of delivery vehicle used for the hair growth compositions.

In a method for enhancing the growth of hair and/or restoring the natural color of the hair in intact skin (scalp), especially including hair of the human scalp, the formulations as described hereinabove are topically applied to the skin tissue to be treated as a liquid or gel at least once

and is left on the skin for a convenient period, generally at least 1-3 hours and preferably for an eight hour period such as overnight. A covering is preferably placed over the gel in order to keep the gel in contact with the treated skin area. To remove the composition, it can be washed off with clear water.

The amount of material which is to be spread on the skin or massaged into the scalp to be treated will be readily apparent. The formulations should be applied in a manner which is cosmetically appealing. In general, in solution or gel form, about 0.5-2.0 cc of formulation is applied per 5-10 cm^2 to the area of the scalp. Depending upon the requirement of hair growth and the extent of baldness, an amount greater or less than 0.5-1 cc of formulation per 5-10 cm^2 of the wound surface may be utilized. In many instances, the depth of the formulation on the skin should be at least about 0.05-0.1 mm. Greater depths may also be used, but generally at the expense of the cosmetic appeal of the product. Obviously, the greater the amount of composition and the greater the depth of gel on the skin surface, the greater will be the delivery of composition to the surface of the skin. In certain cases where the hair is already quite thick, for example, when treating the hair to restore the natural color of the hair, more material may have to be added to the hair in order to assure the individual that a sufficient amount of the composition will come into contact with the underlying skin or scalp area.

In the case of treating hair to restore natural color, the treatment regimen and compositions used are virtually identical to the method used for enhancing the growth of hair. Restoration of hair color generally will occur as an auxiliary result of enhanced growth of hair. Obviously, where the hair color is diminished in color, for example, in gray, graying or even white hair, the methods of use will be the same as if the desired result is enhanced hair growth, but in such a case, the more clearly desired result will be restoration of hair color. Enhanced hair growth most likely will also occur.

In the case of treatment of nails or ungual tissue or

ber of Examples which illustrate some actual tests carried out on skin to enhance the growth of hair utilizing the compositions according to the present invention. It should be understood that the Examples are not exhaustive nor limiting and are presented only for a better understanding of the invention.

EXAMPLES

Materials and Methods

1. Preparation of Hair Growth Gel

The whole procedure was performed under sterile conditions.

a. Delivery System

Four grams of Methyl cellulose (Methocel MC 4000 cp, Fluka AG) in 90 ml of double distilled water was autoclaved.

b. Media

The preferred media contained essential and non-essential amino acids, vitamins, other organic constituents, major inorganic salts, trace elements and buffers and was supplemented with CaCl and L-glutamine and with the non-steroidal anabolic hormones, insulin, thyroxin, growth hormone and insulin-like growth factor (IGF) at the concentrations as indicated below.

Component	Concentration in M
Amino Acids (L-enantiomers)	
Alanine	1.0×10^{-4}
Arginine HCl	1.0×10^{-3}
Asparagine	1.0×10^{-4}
Aspartic Acid	3.0×10^{-5}
Cysteine HCl or Cystine	2.4×10^{-4}
Glutamic Acid	1.0×10^{-4}
Glutamine	6.0×10^{-3}
Glycine	1.0×10^{-4}
Histidine HCl	6.0×10^{-5}
Isoleucine	1.5×10^{-5}
Leucine	5.0×10^{-4}

$SnCl_2$	5.0×10^{-10}
$ZnSO_4$	5.0×10^{-7}
Buffers	
Hepes	2.8×10^{-2}
$NaHCO_3$	1.4×10^{-2}
Phenol Red	3.3×10^{-6}
Supplements	
CaCl2	14.7 ug/ml (Merck)
L-Glutamin	0.877 mg/ml
$NaHCO_3$	1.176 mg/ml
Human Growth Hormone	5 ng/ml (Biotechnology General)
Insulin	5-10 ug/ml (Novo Nordisk) (about 143 mU/ml)
Thyroxine(T_4)	1.0×10^{-7}M (Sigma) (77.69 ng/ml)
Transferrin	5 ug/ml (Sigma)
Sodium Selenite	5 ng/ml (Sigma)
Vehicle	
Methylcellulose (Methocel 4000 cp, Fluka AG)	4% or
Hydroxyethylcellulose	6%

c. Preparation of Hair Growth Formulation

1. 4 grams of the methyl cellulose is autoclaved in 90 ml of double distilled water. The water/methyl cellulose mixture is cooled to 4°C and stirred until the gel dissolves and the solution clears (overnight with a magnetic stirrer in the cold room). To the water/methyl cellulose mixture is added 10 ml of the concentrated MCDB 153 solution (X 10 concentrated media available from Biological Industries, Beth Haemek, Israel) containing the supplements as described above (adjusted to a final volume of 100 ml of formulation). The solution is then mixed well and then decanted into 50 ml sterile test tubes. The formulation is stored at 4°C.

2. Autoclave 6 grams of hydroxyethyl cellulose, middle viscosity 1 (Fluka Cat. #54290) in 83 ml double distilled water. Separately, prepare 10 ml of 10 times concentrated medium containing the supplements as described above (adjusted to a final volume of 100 ml of formulation). Cool the hydroxyethyl cellulose solution to room temperature and stir

6. Do you or anybody else see new growth of hair?

7. if so, how long after you started the treatment did you notice that?

PRELIMINARY CLINICAL TRIALS OF HAIR GROWTH FORMULATION

Patient	Sex	Age	Alopecia Type	Alopecia Duration	Etiology of Defect	Pattern Progress	Dynamics
M.M.	M	25	Diffuse	2 yrs.	Heredity	Combination	Slow
B.A.	M	20	Diffuse	2 yrs.	Male Pattern	Combin.	Rapid
D.M.	M	47	Areata	26 yrs.	Male Patt.	Bald/RHL*	Slow
S.L.	M	40	Areata	15 yrs.	Heredity	Bald/RHL*	Slow
D.T.	M	10	Areata	3 mos.	Infection	Bald pate	Static
E.Z.	M	16	Diffuse	1 yr.	Heredity	Combin.	Rapid
A.W.	M	46	Areata	16 yrs.	Heredity	Bald/RHL*	Slow
M.L.	M	44	Areata	6 yrs.	Male Patt.	Bald pate	Slow
S.P.	M	28	Areata	3 yrs.	Herdity	Bald/RHL*	Rapid
L.L.	F	38	Diffuse	5 yrs.	Unknown	Combin.	Slow
F.B.	F	46	Diffuse	20 yrs.	Heredity	Combin.	Slow
M.G.	F	67	Diffuse	5 yrs.	Heredity	Combin.	Slow
M.L.	F	43	Diffuse	10 yrs.	Unknown	Combin.	Slow
R.G.	F	53	Diffuse	20 yrs.	Unknown	Combin.	Slow
T.B.	F	14	Areata	3 mos.	Trauma	Bald pate	Static

* Receding Hair Line

Table 1
RESULTS OF TREATMENT

Patient	Cessation of Loss	Hair Growth Increase	De Novo Hair Growth
M.M.	1 week	2 weeks	1 month
B.A.	2 weeks	1 month	1 month
D.M.	2 weeks	1 month	2 months
S.L.	2 weeks	2 months	2 months
D.T.	1 week	1 week	1 week
E.Z.	2 weeks	3 weeks	1 month
A.W.	2 weeks	1 month	2 months
M.L.	2 weeks	1 month	1 month
S.P.	2-3 weeks	3-4 weeks	3-4 weeks
L.L.	2 weeks	1 month	1 month
F.B.	2 weeks	1 month	1 month
M.G.	2 weeks	6 weeks	6 weeks
M.L.	3 weeks	6 weeks	6 weeks
R.G.	3 weeks	1 month	1 month
T.B.	1 week	1 week	1 week

In all cases, hair loss stopped within a period of 2-3 weeks. In addition, all of the patients showed an increase in hair growth and all patients experienced de novo hair growth.

Table 2
FURTHER RESULTS OF TREATMENT

Pat.	Sex	Age	HAIR GROWTH None 4m	None 6m+	Slight 4m	Slight 6m+	Moderate 4m	Moderate 6m+	Dense 4m	Dense 6m+
M.M.	M	25					X	X		
B.A.	M	20					X	?		
D.M.	M	47					X			X
S.L.	M	40					X	X		
D.T.	M	10								X Comp.
E.Z.	M	16					X	X		
A.W.	M	46					X	X		
M.L.	M	44					X	X		
S.P.	M	28					X	X		
L.L.	F	38					X	X		
F.B.	F	46					X			X
M.G.	F	67					X			X
M.L.	F	43					X	*		
R.G.	F	53					X	*		
T.B.	F	14								X Comp.

4m - After 4 months of treatment.

6m+- After more than 6 months of treatment.

Comp- Complete cover of the deficit area.

*- Cessation of loss (main complaint).

Example 3
Effects of Hair Growth Formulation on Hair Growth in Mice

To test the efficacy of the instant invention in inducing hair growth in the animal mode, the model of Paus, et al. (1991) was used.

In this experiment, ninety-six (96) C57BL mice aged 5-6 weeks were divided into five groups. Two experimental groups were treated with the instant invention (formulation 1c.2 with transferrin excluded) three times daily by application of the gel to the dorsal depilated area of the mouse. Three control groups were used, two of which were treated with vehicle placebo in the same manner as the two experimental groups. The third group was untreated.

Group No. 3: Treatment with vehicle placebo starting on Day 0 (three times daily) and continued for 28 days.

This group consisted of 20 mice, which were depilated manually on Day 0 of the experiment. On Days 7-8, anagen occurred. On Day 9, cytophosphan (0.5 ml per gram) was administered and on Day 15, catagen occurred. On Days 16-17, anagen was seen and on Day 20 vellus hair was seen. On Day 24, terminal hair was observed and on Day 28, the number of animals that had terminal hair was 4 (mild), 10 (moderate) and 6 (extensive terminal growth).

Group No. 4: Treatment with vehicle placebo starting on Day 9 following cytophosphan injections and continued for 20 days.

This group consisted of 20 mice which were depilated on Day 0 and angagen was observed on Day 9, when the mice were injected with cytophosphan. Catagen was observed on Day 15, and anagen followed on Day 17-18. Vellus hairs were seen on Day 20 and Day 24. On Day 28, terminal hair growth was mild in five naimals, moderate in three animals and extensive in two animals.

Group No. 5: No Treatment given to this control group- used as a baseline for the other studies.

This group consisted of 16 mice, which were depilated on Day 0 and anagen ensued on Days 9-10. On Day 10, cytophosphan was injected and catagen occurred on Days 15-17. On Days 18-21, anagen occurred and on Day 21, punch biopsies were taken.

The various experimental procedures performed on the 5

Table 3
FLOW CHART OF EXPERIMENTAL PROCEDURES

Treated Group	Pre-Inj. 1	Post-Inj. 2	Pre-Inj. 3	Post-Inj. 4	None
Day No.					
0	Depil.	Depil.	Depil.	Depil.	Depil.
1-6					
7	1st Anagen	1st Anagen	1st Anagen		
8		Anagen 60%	Anagen 95%		
9		Anagen 70%	1st Inject.	1st Anag. 100% 1st Inject.	1st Anag.
10	1st Inject.	1st Inject.			Anagen 100% 1st Inject.
11-13					
14		1st Catagen			
15	1st Catagen	2nd Anagen 30%	1st Catag.	1st Catag.	1st Catag. 38%
16	2nd Anagen 50%	Anagen 100%	2nd Anagen 20%	2nd Anagen 30%	Catagen 50%
17	Anagen 100% + 1st CBC + Biopsy	1st CBC + Biopsy	Anagen 100%	Anagen 100%	Catagen 100%
18	2nd Inject.	2nd Inject.			2nd Anagen 100%
19					
20			Vellus Hair	Vell. Hair	
21					Biopsy
22-23					
24			Term. Hair	Term. Hair	
25					
26	2nd Catagen 50%	2nd Catagen 50%			
27	Catagen 100%	Catagen 100%			
28	3rd Catagen 100%	3rd Categen 50%			
29		Anagen 100%			
30	2nd CBC + Biopsy	2nd CBC + Biopsy			

A man in his 40's, who had a diffuse vertex hair loss of 5 years duration has been using the present composition (composition 1c.2) for 5 and 1/2 months. His natural hair coloring is light-reddish brown, but in the vertex area, where hair loss appeared, the color had faded to yellowish-white. After using the present composition, the natural hair coloring of hair in the vertex area was restored. The hair has the same reddish-brown coloring as the res of the hair on the head.

Case #2

A woman in her late 40's suffered diffuse, rapid progressive hair loss throughout the frontal and vertex areas. Application of the present composition for 4.5 months has resulted in cessation of hair loss, new hair growth, increased rate of hair growth and resoration of her original dark-brown pigmentation.

Case #3

A 69 year old female suffered from rapid progressive hair loss in the frontal and vertex areas. She has been using the present composition as recommended for the last 6 months. The treatment has induced cessation of hair loss, increased the rate of hair growth and the growth of new hair. Restoration of her original red pigmentation was observed in hair that has been white for years (notwithstanding the artificial hair rinses she uses).

Case #4

Under Zeiss dissecting Microscope, using a 3mm diameter biopsy punch to delineate the ulcer margins, the corneal epithelium was carefully scraped off the corneas of both eyes.

Experiment 1

Following the injury, the right eyes of 7 animals were treated with the formulation as described above in liquid form using two drops every two hours and in liquid form in the evening. After 24 hours and 48 hours, the eyes were examined under the microscope and photographed.

Experiment 2

Six animals were used in this experiment. Following the injury, in three animals both right and left eyes were treated with the formulation as described above in liquid form using one drop every three hours (until 11 PM and again starting at 6:30 AM). In the other three animals, both eyes were not treated. After 24 hours and 48 hours the corneas of both eyes of all of the animals were examined under the microscope and photographed.

Experiment 3

Six animals were used in this experiment. Following injury, in three animals both eyes were treated with the formulation as described above in liquid form, 2 drops every 2 hours and in gel form, in the evening. In the other three animals, both eyes were left untreated.

2. The mechanism of action of the formulation in enhancing hair growth is partially due to its strong angiogenic activity and increased blood flow.

Example 6
Experiments Performed on Guinea Pigs
Thermal Burn Wounds

Hartley-derived albino female guinea-pigs weighing 250 g were used in this study. The animals were housed in individual cages and fed regular guinea-pig chow and water enriched with Vitamin C ad libitum. All surgical procedures to impart burn wounds to the animals were performed under general anaesthesia of Katamin HCl 150 mg/kg i.m./d,1-2-(chlorophenyl)-2-(methylamino)cyclohexanone hydrochloride, Parke Davis).

The animals were divided into two groups. In the first group, eight guinea pigs were treated with Silverol, which is currently used as a preparation for burn wounds in all military trauma units in Israel. The second group of eight animals was treated essentially with composition 1c1. of the materials and methods section (5 ug/ml insulin, methyl cellulose as gelling agent). The experiment was repeated on three different occasions.

Laser Doppler Flowmeter measurements on burn wounds were performed on days 2 and 8 after injury. Figures 1 and 2 present the data obtained from the Laser Doppler Flowmeter measurements in graph and hologram form. The results evidence that the preferred embodiment evidences effective increase in blood flow compared to conventional therapy. This increased blood flow is believed to be at least partially responsible for the enhanced growth to hair and nail tissue and the unexpected skin revitalization activity these compositions evidence.

While the invention has been described in its preferred embodiment, it is to be understood that the words which have been used are words of description rather than limitation and that

CLAIMS

1. A composition for use in stimulating or enhancing the growth of hair or ungual tissue in intact skin in animals comprising a hair or ungual tissue growth stimulating effective amount of at least one non-steroidal anabolic hormone selected from the group consisting of insulin, triiodothyronine and thyroxine in combination with a minimum essential medium, said minimum essential medium comprising hair or ungual tissue growth stimulating effective amounts of essential amino acids, a mixture of vitamins comprising folate, niacinamide, pantothenate, pyridoxine, riboflavin and thiamin, a mixture of inorganic ions comprising calcium, sodium, potassium, magnesium and chloride and glucose.

2. The composition according to claim 1 further including a hair or ungual tissue growth enhancing effective amount of human growth hormone.

3. The composition according to claim 1 or 2 in the form of a gel produced by including in said composition an effective amount of a gelling agent.

4. The composition according to claim 3 wherein said gelling agent is selected from the group consisting of methyl cellulose and hydroxyethyl cellulose.

5. The composition according to any of claims 2 through 4, wherein the human growth hormone is included at a concentration range of about 0.5 ng/ml to about 50 ng/ml.

6. The composition according to any of claims 1 through 5 wherein said non-steroidal anabolic hormone is insulin included at a concentration of about 50 ng/ml to about 100 ug/ml.

7. The composition according to claim 6 wherein said insulin is included at a concentration of about 500 ng/ml to about 20 ug/ml.

triiodothyronine and thyroxine in combination with a minimum essential medium, said minimum essential medium comprising skin revitalizing effective amounts of essential amino acids, a mixture of vitamins comprising folate, niacinamide, pantothenate, pyridoxine, riboflavin and thiamin, a mixture of inorganic ions comprising calcium, sodium, potassium, magnesium and chloride and glucose.

15. The composition according to claim 14 which includes a skin revitalizing effective amount of human growth hormone.

16. The composition according to claim 15, wherein the human growth hormone is included in a concentration range of about 0.5 ng/ml to about 50 ng/ml.

17. The composition according to any of claims 14 through 16 in the form of a gel produced by including an effective amount of a gelling agent.

18. The composition according to claim 17 wherein said gelling agent is selected from the group consisting of methyl cellulose and hydroxyethyl cellulose.

19. The composition according to any of claims 14 through 18 including insulin at a concentration of about 50 ng/ml to about 100 ug/ml.

20. The composition according to any of claims 14 through 18 including insulin at a concentration of about 500 ng/ml to about 20 ug/ml.

21. The composition according to any of claims 14 through 22 including triiodothyronine or thyroxine at a concentration ranging from about 0.5 ng/ml to about 100 ng/ml.

22. A composition for restoring natural color to hair in intact skin wherein the color of said hair has been diminished, comprising a hair color restoration effective amount of at least one non-steroidal anabolic hormone selected from the group consisting of insulin, triiodothyronine and thyroxine in combination

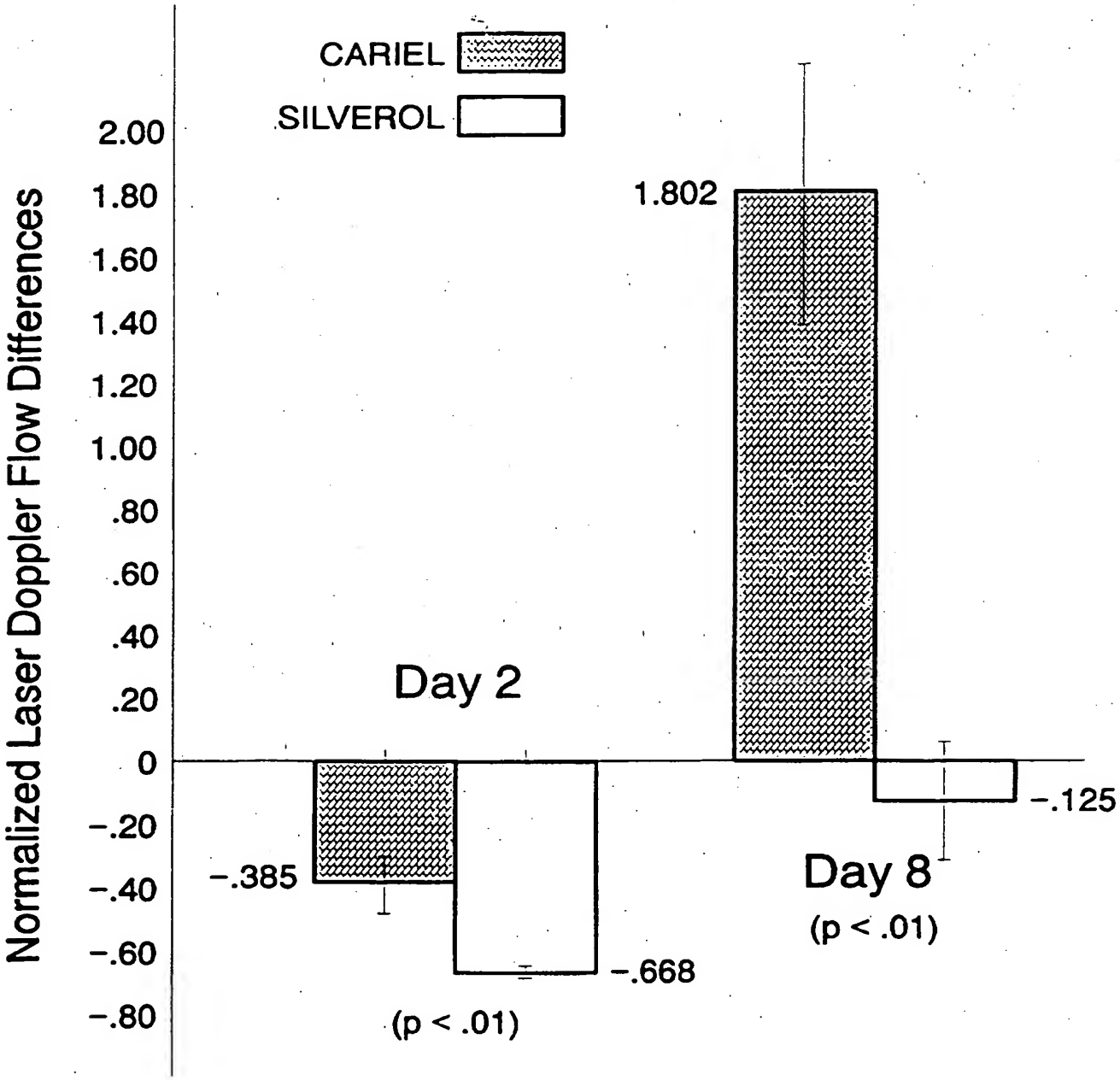
30 wherein said minimum essential medium is a medium selected from the group consisting of ADC-1, Albumin-free LPM, F10, F12, DCCM1, DCCM2, BGJ Medium with or without Fitton-Jackson Modification, Basal Medium Eagle with the addition of Earle's salt base, Dulbecco's Modified Eagle Medium without serum) Glasgow Modification Eagle Medium, Leibovitz L-15 Medium, McCoy's 5A Medium, MCDB 105, MCDB 110, MCDB 202, MCDB 402, MDCB 153, Medium M199 with Earle's salt base, Medium M199 with Hank's salt base, Minimum Essential Medium Eagle, Minimum Essential Medium Eagle with Earle's salt base, Minimum Essential Medium Eagle with Hank's salt base, Minimum Essential Medium Eagle with non-essential amino acids and mixtures thereof.

32. The composition according to any of claims 1 through 31 wherein said medium is MDCB 153.

33. The composition according to any of claims 1 through 32 including an amount of a penetration enhancement agent agent effective for enhancing penetration of said composition through said skin.

34. The composition according to claim 33 wherein said penetration enhancement agent is selected from the group consisting of chondroitin, chondroitin-6-sulphate and dermatan sulphate.

Figure 2



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/02341

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 5-8, 13, 17-21, and 25-34
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.